PROCEDURE FOR COLLECTION AND ANALYSIS OF MARINE AND ESTUARINE SEDIMENT SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT

FOR THE RWQCB 8 SWAMP PROGRAM

(Part of the SWAMP QAMP Appendix G)

Following the methods provided in:

Southern California Bight
1998 Regional Marine Monitoring Survey
(Bight'98)

Field Operations Manual

AND

Quality Assurance Manual

As prepared by:
Southern California Coastal Waters Research Project (SCCWRP)
Westminster, CA

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 2 of 9)

A. Overview

This section provides the Bight'98 QA/QC protocols and requirements for the production of biological data, from sample collection through taxonomic analysis, that will be used in the assessment of benthic infaunal communities from sediment collected from marine and estuarine waters. A laboratory procedures manual has been prepared which describes the acceptable procedures for use in Bight'98 (also part of this Appendix G). Separate procedures are employed by RWQCB 8 in the analysis of samples collected from freshwater systems, as following protocols outlined in the California Lentic Bioassessment Procedure, on file with and available from DFG's Aquatic Bioassessment Laboratory in Rancho Cordova, CA.

Single benthic samples are collected at each station in the survey. Each sample is screened and fixed in the field, returned to one of the participating laboratories, and analyzed for species composition, abundance, and major taxa biomass. The data produced by each laboratory will be aggregated into a single data set and made available for data analysis and interpretation.

B. Sample Collection, Preservation, and Holding

Sediment samples for benthic infaunal analysis will be collected at each station using a SCCWRP-modified 0.1 m2 Van Veen grab or a petite Ponar grab in some situations where the Van Veen grab is too large (Stubbs et al. 1987). The participation of several different vessels and field sampling teams in Bight'98 requires that uniform procedures be followed in the field to ensure high quality samples and consistent results. Field personnel will be provided with the Field Operations Manual (1998) and instruction on sampling procedures, application of sample acceptance criteria, sample processing, and use of field data forms. All personnel are expected to understand and properly carry out all steps in the collection, screening, relaxation, and fixation of infaunal samples, and the subsampling and handling of sediment chemistry and toxicity samples.

Capability will be established by means of field audits by the Field QA Specialist prior to sampling for the survey. During the field audits, the QA Specialist will provide corrective instruction as necessary. The Field QA Specialist (or designee) will also conduct subsequent audits on benthic sampling procedures during the Bight'98 survey to assure that sampling is conducted in a uniform manner and all required information is recorded by all field crews.

A Measurement Quality Objective (MQO) of 90% has been established for completeness of the field collection of benthic samples. This completeness goal was established in an attempt to derive the maximum statistical power of the

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 3 of 9)

sampling design. The MQO was not set at 100% in recognition that the randomized selection of sampling sites employed in the Bight'98 survey is likely to result in the selection of some sites where Van Veen or Ponar grab sampling will be difficult or impossible. Nevertheless, field crews are expected to strive to meet or exceed this MQO. To this end, site acceptability criteria and relocation procedures are provided in Section VII, and sample acceptability criteria and minimum sampling effort are stipulated in Section 9 of the Field Operations Manual. As many as nine attempts at a site must be made to meet the site acceptability criteria. Once a site has been accepted, a minimum sampling effort of four attempts to collect an acceptable sample is required at each station.

Sample acceptability criteria have been established in the Field Operations Manual (1998) based on sample condition and depth of penetration of the grab. An acceptable grab is characterized by an even surface with minimal disturbance and little or no leakage of overlying water, and a penetration depth of at least 5 cm, if the target depth of 8 cm cannot be achieved. Samples not meeting these criteria are rejected.

In the laboratories, samples will be stored in a safe and secure manner protected from environmental extremes. Exposure to temperatures above 30C should be avoided so as to retard evaporative loss. Do not refrigerate samples containing formaldehyde as paraformaldehyde will be formed at lower temperatures. Samples are to be transferred from fixative (borate-buffered 10% formalin) to preservative (70% ethanol) after 72 hr (but within two weeks) of collection. When transferring, thoroughly wash the fixative from the sample, using a 0.5 mm (or smaller) mesh screen to avoid specimen loss. Stored samples must be periodically inspected to assure that the closure is tight and the preservative level adequate. If evaporative loss of preservative is evident, top-off the sample using 100% ethanol.

C. Laboratory Operations

The laboratory analysis of infaunal samples for the Bight'98 involves three processes: sample sorting, biomass estimation, and organism identification and enumeration. Quality assurance in the form of procedures and standardized reporting requirements are provided in the Infaunal Sample Analysis Laboratory Manual for all three processes. The QA Specialist (or designee) will conduct audits of each laboratory while sample analysis is underway to assure that the Bight'98 procedures are being followed. For the most challenging process, organism identification, additional quality assurance steps are included in order to foster comparability among the taxonomic data sets produced by the four participating laboratories. The quality assurance steps for taxonomic analysis are discussed separately below.

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 4 of 9)

Sample sorting

Quality control of sorting is essential to assure the value of all the subsequent steps in the sample analysis process. Sample material is sorted into six taxa lots: annelids, mollusks, arthropods, ophiurans, ophiuroids, miscellaneous echinoderms, and "other phyla". A standard sorting form is used for tracking the sample. It includes the name of the laboratory and technician responsible, time required for sorting, number of taxa lots and sample containers, and comments.

Re-sorting of samples is employed for quality control of sorting. Each laboratory participating in the survey has an existing re-sorting protocol for this purpose. All share a minimum re-sorting effort of 10% of the material sorted with a minimum acceptable removal efficiency of 95%, the equivalent of an accuracy MQO of 5%.

Two approaches are used for re-sorting. In one, a 10% aliquot of every sample processed by a sorter is resorted. In the other, 10% of the samples processed by a sorter are completely resorted. In both cases, all re-sorting is conducted by an experienced sorter other than the original sorter. For the Bight'98, either of the two approaches is acceptable. The re-sort method used is noted on the sorting form Quality Control Report section of the Sorting form along with results.

Percent sorting efficiency is:

$$\frac{\text{Number of Organisms originally sorted X 100}}{\text{\# of Organisms originally sorted + \# found in resort}}$$

If sorting efficiency is greater than 95%, no action is required. Sorting efficiencies below 95% will require re-sorting of all samples sorted by that technician and continuous monitoring of that technician until efficiency is improved. Actions taken are to be described on the Quality Control Report section of the Sorting form and the report signed by the responsible supervisor. Organisms found in the resort should be added to the original data sheet and, if of significant biomass, included in the sample biomass estimation. Once all quality control criteria for sample sorting have been met, the sample debris may be is discarded.

Taxonomic analysis

The goal of taxonomic analysis for Bight'98 is species level identification of all macrobenthic organisms collected and an accurate count of each species. This task is complicated by the participation of several laboratories in this analysis. The challenge of achieving accurate and consistent results inherent in a large survey of infaunal organisms is compounded by differences in expertise,

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 5 of 9)

experience, and opinion of the many taxonomists involved in the analysis.

The Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) is cooperating with Bight'98 to provide an important element of quality assurance for this aspect of the project. SCAMIT is a regional organization of taxonomists, many of whom are primarily involved in infaunal monitoring studies of wastewater impacts within the southern California Bight. SCAMIT was founded in 1982 with the goals of promoting the study of marine invertebrate taxonomy and developing a regionally standardized taxonomy for use in environmental monitoring studies. Activities center on cooperation and communication among the region's taxonomists, sharing of expertise, and monthly workshops. Results of the workshops and other information is communicated to the membership through a monthly newsletter.

SCAMIT's cooperation includes the provision of standards for nomenclature use and a mechanism for mutual assistance and exchange of information among the taxonomists involved in Bight'98. The taxonomic nomenclature used in Bight'98 follows the SCAMIT hierarchical species listing (SCAMIT 1994). This list represents a consensus for standard usage of taxa names in POTW monitoring programs in the Bight. In addition, SCAMIT protocols for the use of open nomenclature (SCAMIT 1986) are followed. Taxonomists from the participating laboratories are required to participate in special SCAMIT/Bight'98 workshops prior to the sampling period that focus on the taxonomy of groups requiring particular review to promote uniform treatment in the upcoming survey. Pre-survey workshops consider nemertea, platyhelminths, and other groups. The workshops provide training, pooling of regional resources, and designation of the local expert(s) to be called upon for assistance during sample analysis.

A pre-qualification exercise will be performed in order to assure comparability among laboratories identifying infaunal samples. Each organization will provide a list of taxonomists and their specialty areas. Taxonomists who were not involved in the 1994 survey will be sent two samples for ID. The results for number of taxa, number of organisms, and accuracy of the ID will be scored by a committee using procedures described in Montagne and Bergen (1997). If the results meet the minimum quality objectives (MQO), the taxonomist will be considered qualified. Otherwise, the benthic group will bring a recommendation on qualifying the taxonomist to the Steering Committee.

After sample analysis has begun, SCAMIT/Bight'98 workshops will continue at least monthly to address taxonomic problems arising during analysis of the Bight'98 samples. A process for integrating these workshops into the sample analysis process is described in the Infaunal Sample Analysis Laboratory Manual (Figure 6-1). Protocols for the erection and documentation of provisional species

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 6 of 9)

names, based largely upon SCAMIT recommendations (SCAMIT 1986), are provided in the Bight'98 Laboratory Manual. These protocols are intended to assure that adequate documentation is created for any provisional name erected and that the information is quickly and efficiently communicated to all participating taxonomists.

The series of SCAMIT/Bight'98 workshops will culminate in a synoptic review of the data set compiled from all laboratories, and investigation of possible inconsistencies revealed in that process (including examination of voucher specimens or sample lots as needed for resolution). This review also draws upon the results of the quality control re-analysis of 10% of the samples analyzed by each laboratory.

While the SCAMIT/Bight'98 workshops are the primary means for exchange of information and assistance, the taxonomists participating in analysis of Bight'98 samples should maintain frequent and informal interaction throughout the process. The use of the Bight'98 bulletin board established for this purpose is encouraged.

The creation and maintenance of voucher collections is an essential element of the QA/QC process. A voucher collection is an invaluable tool during the course of the study, when access to voucher specimens greatly assists the taxonomists in avoiding inconsistent identifications. Upon completion of the study, voucher collections provide other workers the means to determine the identity of species as understood by the original taxonomist. Each participating laboratory must create a voucher collection of all species identified in Bight'98 samples analyzed in that laboratory. Procedures for the creation, maintenance and documentation of the voucher collections are provided in the Infaunal Sample Analysis Laboratory Manual. These collections are separate from the laboratories' existing voucher collections and will be the source of material from which is drawn a common Bight'98 voucher collection upon completion of the survey. These collections provide material for review during SCAMIT/Bight'98 workshops and the synoptic review of the data upon completion of analysis.

The ultimate repository of the Bight'98 voucher collection and sample material has not yet been identified. This decision will have to balance the need to have the vouchers & sample material properly cared for; and the need to have the material easily available for subsequent review or re-analysis. Taxonomists involved in subsequent regional monitoring efforts will want access to the pilot project sample material. This access makes it possible for the taxonomist to re-identify taxa lots as appropriate to maintain the integrity of the original survey (see SCAMIT Comments & Recommendations to the Monitoring Sub-Committee of the Southern California Bight Review Committee, Jan 1998). SCCWRP's central role in the project as well as its central location makes it the logical repository of

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 7 of 9)

the sample material. This would require SCCWRP to make a long-term commitment to the maintenance of such collections, including curatorial care and management of future access. If this commitment cannot be met then other alternatives (e.g., natural history museums) will have to be explored.

Quality Control of Taxonomic Analysis

While the quality of taxonomic analysis in Bight'98 relies heavily on the measures described above, quality control is also provided by the re-identification of 10% of the samples processed by each laboratory. Re-identification will be conducted at a participating laboratory other than that which originally analyzed the samples. Samples for re-identification are selected randomly from each lab's assigned set of samples and randomly re-distributed to the other three laboratories. Results are reported on standardized re-identification sheets. The taxonomists conducting the re-identification do not have access to the original results.

The results are returned to the originating lab where the original two sets of results are compared recorded on the re-identification sheet and a standardized comparative report of results discrepancies is prepared. Discrepancies are identified and the report returned to the lab responsible for the re-identification. The two laboratories attempt to reconcile discrepancies. In the process, apparent error is discriminated from actual error and the number of each type of error recorded. Apparent errors are cases where the discrepancy is a result of a difference in the level of the identification, rather than a misidentification. For example, the discrepancy between a report of Tubulanus sp. and Tubulanus frenatus does not represent an error, but rather a decision by one taxonomist to identify the specimen only to genus level. This decision may be based on the taxonomist's judgment that the specimen's condition is too poor for a species identification, or may reflect his or her lack of expertise in this particular group of organisms. In the latter case, the difference in treatment provides a indication where assistance from other taxonomists involved in the Bight'98 is needed. Nomenclature differences are also examples of apparent error. Examples of real error are misidentifications and miscounts. In addition to characterizing analytical accuracy, this process provides information for the SCAMIT/Bight'98 synoptic review of the data compiled from the four laboratories at the end of the survey. Significant discrepancies in count are resolved by a third count.

A MQO of 10% has been established for the accuracy of taxonomic analysis of infaunal samples. After reconciliation of differences, the percent accuracy for the sample is calculated by the formula below. The calculation considers real errors only. The number of counting errors is based upon the difference between the original count and the resolved count.

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 8 of 9)

$$\frac{\text{Number of Organisms in QC resolved recount - Number of errors}}{\text{Number of Organisms in resolved count}} \times 100$$

The following types of errors are included in the total number of errors:

· Counting errors (e.g., counting eleven individuals of a species as 10, including dead bivalves in a count); · Identification errors (e.g., identifying species X as species Y where both are present); · Unrecorded taxa errors (e.g., not identifying species X when it is present). · Recording errors (e.g., recording species X as species Y by recording on the wrong line on a pre-printed data entry sheet).

Each contributing laboratory must maintain an identification and enumeration accuracy of 90% or greater. If accuracy falls below this level, the taxa lot(s) contributing most to the error are singled out. These taxa lots in the preceding or next five samples analyzed by that laboratory (or taxonomist) must be re-analyzed. If the errors are found to be systematic, those taxa lots in all samples processed by that laboratory (or taxonomist) must be re-analyzed. The taxa lot(s) in which substantial error is found must be re-identified in all samples analyzed by the original laboratory. The calculated accuracy is reported on the Quality Control Accuracy Report, as well as any actions required. The completed report is signed by the responsible supervisor.

D. Information Management

Sample tracking

Each Laboratory will provide a means of sample tracking within their laboratory. The sample tracking process must include documentation of receipt of samples, assurance that sample storage procedures are followed and that required tracking information is transmitted to the Information Management Officer.

Record keeping and reporting

Each laboratory must be responsible for maintaining thorough and complete records through all stages of the sample analysis and QC procedures. Each laboratory will employ its own bench sheet for taxonomic analysis. For Bight'98, certain standard forms of notation are employed with the taxonomist's bench sheet that assure that all labs collect the required formation in a uniform fashion. Standardized forms are used for sorting, biomass estimation, and all QC checks. Each participating laboratory will retain its taxonomic bench sheets and voucher sheets.

All QC reports will to be submitted with the analytical results. Copies of all these documents are to be retained by the individual laboratories. Copies of all quality control reports are to be provided to the Quality Assurance Coordinator.

SAMPLE COLLECTION PROCEDURES FOR SAMPLES FOR BENTHIC INFAUNAL COMMUNITY ASSESSMENT USING SEDIMENT FROM MARINE AND ESTUARINE WATERS
(Page 9 of 9)

The laboratory manager supervisor is responsible for assuring that all steps in the process of analyzing infaunal samples follow Bight'98 procedures and that all QC steps are completed and documented. The manager supervisor must implement any specified corrective actions resulting from QC protocols. He or she is also responsible for preparing their data and documents for transmission to the Information Management Officer in the proper form. All data entry must be subject to the established transcription error checking procedures within the originating laboratory.